Neutron and γ-Ray Radiation Killing of *Bacillus* Species Spores: Dosimetry, Quantitation, and Validation Techniques

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On the cover: Scanning electron micrograph of a dry *B. pumilus* spore strip (8,400X) demonstrating the hollow cellulose fiber network and spores attached. Boxed area shows detail enlarged in Fig. 1b.

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Contents

Abstract

The validation of sterilization processes is currently monitored by qualitative estimates of bacterial spores using inactivation of commercially available spore-impregnated cellulose fiber strips where sterility is determined by the presence or absence of growth in an appropriate medium. Using the standardized technique in the U.S. Pharmacopeia (USP) National Formulary XXII, spore kill is quantified by pulping multiple spore strips in a large volume of diluent. Such tests do not allow quantitative analysis of killing on each separate strip. In this study, the standard USP test was modified to quantify sterilization of spores on single cellulose fiber strips that were irradiated with fission neutrons produced by a TRIGA reactor and by ^{60}Co γ-rays. The technique, developed at the Armed Forces Radiobiology Research Institute (AFRRI), was validated for similarly treated spore strips by comparing data obtained from an independent commercial laboratory and certificated values provided by the supplier of the spore strips. The number of *Bacillus subtilis* and *Bacillus pumilus* spores identified by the two laboratories were similar for controls (unirradiated spore strips) and for spore strips that received selected doses of fission neutrons or γ-rays. It was thus concluded that assay techniques, developed and used at AFFRI to enumerate spore survival, accurately quantify spore kill after neutron and γ-irradiation. These techniques can be used in more extensive studies to determine the killing effect for spores irradiated under a variety of environmental conditions.

Introduction

Vegetative bacteria and bacterial spores are killed by various types of ionizing radiation in a dose-dependent manner. In the health and food industries, ionizing radiation is used to sterilize items such as foodstuffs, packaging material, and surgical instruments (for reviews written within the last 12 years see references 11, 13, and 15.) In basic research studies, bacterial spores have been used as "simple" life forms to determine the number and size of sensitive "targets" that require inactivation before measurable damage or death occurs after irradiation and to argue for the direct or indirect action of radiation on sensitive targets within the organism (7). Various types of radiation have been used to determine the size of the sensitive target(s) in spores (4, 12).

Sterilization effectiveness is related to a specific radiation type. The sensitivity of vegetative or spore forms of bacteria to radiation is measured in D_{10} values; the D_{10} is the absorbed dose required to reduce the number of viable spores to 10% of its original number (i.e., 90% of the spores have been killed). The units of D_{10} are conventional units of absorbed radiation dose: rad or gray (Gy) (100 rad equals 1.0 Gy). The D_{10} value should not be confused with the D_{37} value, which is the dose of radiation necessary to reduce the size of the population in question to 37% of its original number.

Although bacterial spores are generally more resistant to radiation than vegetative bacteria, there are exceptions. For example, Silverman (15) reports that vegetative *Deinococcus radiodurans* (formerly *Micrococcus*) and *Moraxella osloenis* have D_{10} values to γ-radiation of 0.22 Mrad and 0.58 Mrad, respectively, and spores of *Bacillus subtilis* and *Bacillus pumilus* have D_{10} values to γ-radiation of 0.06 Mrad and 0.4 Mrad, respectively. It is interesting to note that survival of *B. subtilis* spores impregnated in cellulose fiber strips is routinely used to determine effectiveness of dry heat sterilization, and that survival of *B. pumilus* spores impregnated in test strips is used to determine sterilization effectiveness after high doses of radiation of various types.

Complete sterilization or neutralization of bacterial spores has been studied for a variety of radiation sources. Electrons, γ-rays, x-rays, and ultraviolet radiation were used to determine killing of spores (13, 15). Heavy charged particles were used to investigate the amount of damage produced in spores exposed in various types of gas environments and to estimate the number of target sites and hits needed to kill the organism (5, 12).

Killing spores and vegetative bacteria by neutrons was investigated at a time when dosimetry techniques were not well established (8, 16). To date, killing spores by neutron irradiation has not been studied where modern dosimetry techniques are employed.

In this study, radiosensitive *B. subtilis* spores and radioresistant *B. pumilus* spores, dried on filter paper strips, were both separately irradiated with high doses and dose rates of fission neutrons and γ-rays, respectively. The aims of this study were to develop (1) a technique to accurately quantify the spores killed, as measured by radiation kill curves for neutrons compared to γ-rays, and (2) techniques to accurately identify radiation doses delivered to spore samples, specifically those spore

samples irradiated with fission neutrons. A related paper will present extensive survival data for these species of bacterial spores irradiated in dry and wet states.

Materials and Methods

Spores

B. subtilis and *B. pumilus* spore-impregnated cellulose fiber strips were obtained from Raven Biological Laboratories, Omaha, NE. The strips were made of Schleicher and Schuell #470 filter paper (6 mm wide, 38 mm long, and 1 mm thick) and contained a bioburden of 1 x 10^8 *B. subtilis* spores [American Type Culture Collection (ATCC) #9372] or 1.3 x 10^8 *B. pumilus* spores (ATCC #27142) . A certificate of quantitative analysis was supplied for each lot of bacterial spore-impregnated strips. Before and after irradiation, packages containing the spore strips were stored with Drierite (W. A. Hammond Drierite Co., Xenia, OH) in a dehumidified environmental chamber housed in a refrigerator maintained at 4° C.

Preparation of Dry Spores

Each cellulose fiber strip of bacterial spores was aseptically removed from its envelope; 8-10 strips were packed into each round-bottom 12 x 75-mm sterile polystyrene tube, an effective container for irradiation. Before packing the spore strips, sterile cotton-type dressing sponges were forced into the bottom 30 mm of each tube. This material, having radiation absorption and scattering characteristics equivalent to spore strips, displaced air in the bottom of the tube. Placement of the spore strips at the top of the cotton plug also permitted reproducible positioning of the spore strips within the tubes for irradiation.

Gamma Irradiation

Spore strips in the polystyrene test tubes were loaded into a single tiered, 7.5-cm-high and 15-cm-long 10-tube lucite holder and were irradiated in a bilateral γ-radiation field in AFRRI's ^{60}Co facility for high dose irradiation (2). Charged particle equilibrium within the spore strips in the tubes was achieved by irradiating the whole specimen package through 6-mm-thick lucite walls and by placing "blank" tubes prepared as indicated below. A 0.5-cc tissue-equivalent ionization chamber with calibration traceable to the National Institute of Standards and Technology was used

for dosimetry. The dose rate at the irradiation position was 168.4 Gy/min. The tissue-air ratio was 0.95, and the radiation field was uniform to within ±2%. Dosimetric measurements were made in accordance with the protocol of the American Association of Physicists in Medicine (1) for determining the absorbed dose from high-energy photon and electron beams. After the required dose of radiation was delivered to a specific tube containing spore strips, the tube was removed within 5 min and replaced with a similar test tube containing Schleicher and Schuell #470 filter paper strips without spores to ensure a similar scattering environment for the remaining samples that were additionally irradiated to accumulate higher doses. Unirradiated "control" spore strips were packaged in test tubes as described earlier and maintained in a radiation-free area. The temperature in the exposure room and the radiation-free area was maintained at 21±1° C. Irradiated and control unirradiated spore strips were stored with Drierite in a refrigerator as described above until processed.

Fission Neutron Irradiation

Spore samples were irradiated with fission neutrons in AFRRI's Training, Research, and Isotope, General Atomics (TRIGA) Mark-F reactor (10). The reactor has a moveable core suspended in a large pool of water. During irradiation, the core was positioned at the pool end adjacent to the semicylindrical reactor tank wall that projects into the exposure room. In this configuration, neutrons streaming from the core pass through minimal moderating material—less than 2.5 cm of water between the core and the reactor wall.

The exposure array for neutron irradiation consisted of a lucite holder (23 x 7.5 x 7.5 cm) in which up to 12 sterile polystyrene test tubes (12 x 75 mm) containing spore strips were suspended in a single row between two 3.25-mm-thick lucite restrainer walls. Test tube slots on each end were reserved for "blanks" to ensure similarity of scatter to all test tubes. The exposure array was placed in a lucite harness that passed through an aluminum extractor tube into a bismuth cave designed to reduce the γ component of the total dose given to the spores. The cave was constructed with 26 x 16.5-cm bismuth bricks of different thicknesses, in the range of 3.2 to 4.0 cm. Bismuth bricks were stacked in front of the reactor tank wall to shield spore samples positioned in the extractor tube from γ rays that emanated from the core. Additional bricks enclosed the extractor tube portion containing the exposure array and spore samples during irradiation to shield from γ radiation that scattered from exposure room walls. Samples were transferred between the bismuth cave and a preparation area outside the exposure room through the aluminum extractor tube that penetrates the exposure room walls. A motorized pulley system,

controlled from the preparation area, was used for insertion and withdrawal of sample arrays within 5 min. After a required radiation dose was delivered to a tube of spores, the exposure array was withdrawn, and the sample tube was removed and replaced with a "blank" tube containing Schleicher and Schuell gel blot paper GB002 on a weight-equivalent basis. Remaining spore-containing tubes were sent back into the bismuth cave via the pulley system to receive additional radiation. This ensured the same scatter environment for all tubes of spores during irradiation. Temperature in the exposure room during radiation was maintained at 25 ± 2° C.

Before irradiation of spore strips in the neutron field, radiation dose rates were measured with paired-ionization chambers (6, 9). Ionization chambers used were a 0.5-cc tissue-equivalent chamber and a 0.5-cc magnesium chamber, each with a calibration traceable to the National Institute of Standards and Technology. The midpoint of the array within the bismuth cave was 61.5 cm from the tank wall and 123 cm above the exposure room floor. At this position the neutron to total dose ratio was 0.95 ± 0.2 for all reactor runs, and at a steady state reactor power of 500 kW the dose rate obtained was 46.2 Gy/min. Neutron field uniformity was determined by sulfur pellet activation and was within ±6% across the length of the array. In a similar configuration with a lead cave (instead of the bismuth cave), fluence-weighted mean energies were 0.71 MeV for neutrons (14) and 1.8 MeV for γ-rays (19). The exposure for each irradiation was monitored with ionization chambers and fission ion chambers located at fixed points in the exposure room. These monitor chambers are always calibrated during dosimetry runs performed at the beginning of every reactor operation.

Quantification of Spore Survival

The technique for quantifying total viable spores was adapted from the USP Monograph 1035, National Formulary XXII, 1990, pp. 170-175. Techniques and modifications used in this investigation are given below. Modification was validated in experiments wherein cellulose strips containing spores from both bacterial species were irradiated and underwent simultaneous evaluation at AFRRI and at an independent commercial laboratory operated in compliance with current Good Manufacturing Practices (GMP). In these experiments, the independent commercial laboratory performed single spore strip analyses by pulping the strip in 100 ml of sterile deionized water (diluent). AFRRI performed the analyses as indicated below.

Step 1: Pulping of Dry Spore Strips. Single dry spore strips were pulped in 25-ml of chilled sterile diluent in a 35-ml chilled sterilized blender cup for 3 min on low speed in a blender (Waring Blender, Model No. 700B, New Hartford, CT) operated at 7200 rpm and controlled by a variable rheostat (Model 3PN116C, Superior Electric Co., Bristol, CT). Homogeneity of pulping was ensured by periodically examining the sharpness of the blender cutting blades. Pulping speed was controlled to avoid killing spores by excessive heat. In trial pulping procedures, microscopic appearance of the mixture of pulped cellulose fibers and spores was determined using methylene blue dye. Selected irradiated and nonirradiated, nonpulped, dry spore-impregnated strips were examined using bright light, scanning, and transmission electron microscopy.

Step 2: Heat Activation of Spores. Spores were heat activated to enhance germination and to kill vegetative bacterial contaminants on the pulped cellulose strip. Before heat activation, pulped cellulose spore strip media suspensions were aspirated several times by repetitive pipetting to ensure homogeneity. Ten milliliters of suspension were transferred from the blender cup to a sterile screw-capped tube (16 x 123 mm). The tube and its contents were kept on ice up to 1 hr before actual heat activation. Pulped spore strip media suspensions could be stored up to 18 hr (overnight) in a refrigerator at 4° C without losing viability.

A 15-min heat activation of suspensions was performed in a water bath maintained at 65–70° C. Activation timing started when the temperature of the suspension reached 65° C. Temperature of the suspension was monitored with a thermometer placed in a similar tube containing 10 ml of water.

Subsequent to heat activation, the suspension and the monitoring tube with thermometer were cooled rapidly in an ice bath to 0-4° C. Spore suspensions were then ready for dilution.

Step 3: Serial Dilutions of Spore Suspensions. Serial dilutions of spore suspensions were prepared by transferring two 1-ml aliquots to two tubes containing 9 ml of chilled sterile deionized water. A single pulped cellulose spore strip was thus analyzed in two parallel series, which controlled for dilution errors. Tenfold serial dilutions were prepared from each 1-ml aliquot to a level where 30-300 colonies could be counted. An exception to this was the plating of all pulped material to determine if the highest radiation doses, (doses that reduced the spore load by 6 logs

or more) had completely killed all spores. The number of dilutions needed was determined empirically and related to the dose and type of radiation.

To ensure that all spores were released from the filter fibers and that all clumps of spores were disaggregated, the tubes containing suspensions were vortexed 10 times before proceeding with dilutions. Serial dilutions were prepared by vortexing each tube 3 times before transferring to the next tube.

Step 4: Pour Plating of Appropriate Dilutions. Tubes containing each suspension were vortexed once before removing a 1-ml aliquot. Pour plates were prepared from each dilution series by placing 1-ml of each selected dilution in each of two 15 x 100 mm petri dishes.

Within 20 min after deposition of test material, 20 ml of sterile 4.0% BBL soybean casein digest agar medium (Trypticase Soybean Agar (TSA) 11043) were added to each plate. Multiple lots of TSA obtained from Becton Dickinson Microbiology Systems, Cockeysville, MD, were used throughout this study. The autoclaved TSA was cooled to 45-50° C in a water bath before pouring to avoid killing heat-activated spores. Agar-spore plates were swirled in a figure-eight fashion four times to attain a homogeneous dispersion.

Step 5. Pour Plating for "Tailing" after High Radiation Doses. Survival curves of spores show an exponential reduction to increasing radiation doses except at the highest levels of radiation where survival comprises a very small residual number of spores—a "tailing" off of the curve (3). In this study, when the number of surviving spores (*B. pumilus*) in the pulped suspension after high γ-radiation doses was expected to be fewer than 30-300/plate, the entire 25-ml contents of the blender cup was heat shocked in a 50-ml tube to ensure recovery of all viable spores. The temperature was monitored by using a thermometer in a similar tube containing 25 ml of water. After heat shock, the entire suspension was plated in 5-ml volumes on five 20 x 150-mm sterile plastic Petri plates. The number of colonies on each plate was totaled to give the total viable colony-forming units per strip.

Step 6: Incubation of Plates. All pour plates were allowed to solidify at room temperature for up to 2 hr on a flat laboratory bench surface. Incubation was for 24 and 48 hr in an inverted position at 30-35° C in air. Pour plates not inoculated with

spore suspensions or diluent were incubated to check for sterility of the media. Diluent was also plated to check for sterility.

Step 7: Counting of Colonies. It was assumed that single viable spores produced vegetative progeny with unique colony characteristics. Colonies were counted after 24 and 48 hr of incubation. Counts at 48 hr were used to calculate numbers of viable spores per strip. Counts at 24 hr were used to monitor accuracy of 48-hr counts and to monitor counts obtained by different investigators. Colonies were counted using a Fisher Accu-Lite Colony Counter with 4X magnification (Model 133-8002, Accutech Corp., Brooklyn, NY). The average number of spores per strip was determined by multiplying the appropriate dilution factor times the number of colonies counted. When bacterial colonies grew on uninoculated control plates or plates inoculated with sterile diluent, companion plates used to test for spore neutralization by radiation were invalidated.

Identification of Vegetative Colonies Formed by Spores

In a limited number of instances, vegetative bacterial colonies formed from unirradiated, neutron-irradiated, and γ-ray-irradiated spores were evaluated for colony characteristics and uptake of Gram's stain 48 hr after incubation. Colonies surviving after the highest doses of radiation and colonies having "questionable" morphologies in comparison to unirradiated spores were also submitted for Gram's staining.

Data Reduction and Statistical Analyses

Each pair of plates from each dilution series was evaluated for dispersion homogeneity and suitability for counting. Dilution plates with dispersion homogeneity and colony numbers up to 300 were used to determine spore survival. Dilution plates not meeting these criteria were not used for this determination. In 99% of cases, a total of 4 agar plates (2 plates for each series) for each dilution of each strip were counted, and an average value was calculated for each dilution for each strip. At each dose and type of radiation, 2-5 strips were evaluated, and a mean and standard error of the mean for these values computed. The survival fraction for each dose and type of radiation was then calculated by dividing this mean by the mean number of viable spores determined from unirradiated control strips ($n = 10$-13). The standard error of this survival fraction was determined using error propagation and the standard errors of the irradiated and control means. The survival fractions and their standard errors were plotted as a semilog ($\log_{10}$) plot of survival fraction versus radiation dose.

At the highest radiation doses where substantial spore kill was noted (spore bioburden reduced by 6 logs or more), colonies on dilution plates were enumerated and/or identified to determine if tailing occurred, 100% kill had occurred, or bacterial contaminants had grown on plates.

Validation of Revised Test Procedure

In initial experiments and within 48 hr of irradiation, spore strips were divided into two lots for analyses. One lot, in a temperature-controlled container containing Drierite, was released and transmitted by special courier from AFRRI to a commercial laboratory for testing. The second lot was maintained in AFRRI under the storage conditions in Materials and Methods. Single strips were analyzed at the commercial laboratory within 2 weeks after irradiation by following USP standardized techniques (17). Single strip analyses at AFRRI were prepared as described above in Data Reduction and Statistical Analyses. Data from the two laboratories were compared against certificated values obtained from Raven Biological Laboratories (Omaha, NE). Statistical analyses of data sets from both laboratories indicated no differences in final spore concentrations on the cellulose fiber strips.

Results

Microscopic Appearance of the Cellulose Fiber-Spore Mixture

Methylene blue stains of blended pulp mixtures examined under 400X (Leitz Dialux microscope) indicated that the spores were not all released from the cellulose fibers. Aggregates of fibers were observed in some cases. No apparent culturing problems were associated with these observations—vegetative colonies did not appear to overlap each other on the agar plates. In addition, the small standard errors associated with the data points seen in our figures are consistent with the high degree of precision in the clonogenic aspects of the assay.

Light microscopy of selected dry spore strip samples revealed that spores were not only distributed on the outer surfaces of the strips but dispersed in an uneven manner

Fig. 1a. *Scanning electron micrograph of a dry* B. pumilus *spore strip (8,400X) demonstrating the hollow cellulose fiber network and spores attached. Bar = 5* μ*m.*

throughout the entire cellulose fiber matrix. Scanning electron microscopy (Figs. 1a and 1b) showed spores attached to the surface of fibers and lodged in the fiber crevices. Additional transmission electron microscopy (data not shown) indicated no visible difference between irradiated and nonirradiated spores.

Fig. 1b. *Detail of boxed area in Fig 1a.*

B. subtilis and *B. pumilus* Colony Morphology

B. subtilis spores produced vegetative surface colonies with undulate margins and smooth surfaces and were white or sometimes tan in color. *B. pumilus* surface colonies were undulate, smooth, and light tan, becoming more lobed with age. Subsurface colonies of both species were smaller than surface colonies, disc shaped, and usually became lobed with time.

No differences in colony characteristics were seen between nonirradiated, neutron-irradiated, and γ-ray-irradiated spores. At high radiation doses there were some colonies that appeared to have lost the lobed characteristic.

Validation of Revised Test Procedure

Surviving fractions of *B. pumilus* and *B. subtilis* spores determined by AFRRI and the independent laboratory after γ-ray and neutron irradiation were used to validate the modified USP XXII protocol (17). In the first set of survival validation studies done with *B. pumilus* (Fig. 2), γ-ray doses up to 15 kGy resulted in a 6-log kill curve with no apparent shoulder. There were no differences in numbers of spores killed at all radiation doses as determined by the two laboratories. Both laboratories recorded "tailing" at the two highest radiation doses, the variability of which seemed related to the scant number of colonies recorded.

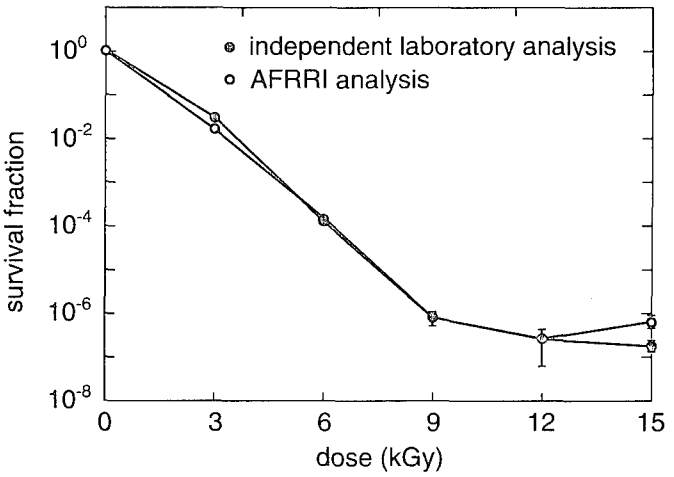

Fig. 2. *Survival fractions of dry* B. pumilus *spores after selected doses of γ radiation; validation of the AFRRI technique used for spore survival analyses after γ-irradiation compared to analyses performed by the independent commercial laboratory. The filled circle (●) represents survival data determined by the independent commercial laboratory, and the open circle (O) represents survival data determined by AFRRI. Each data point represents spore survival obtained from analyzing 2-11 cellulose fiber spore-impregnated strips.*

In a second set of limited survival validation studies done with *B. pumilus* (Fig. 3), neutron radiation doses up to 2 kGy resulted in a 3-log kill curve with no apparent shoulder and no "tailing." There were no differences in numbers of spores killed by the radiation doses as determined by the two laboratories.

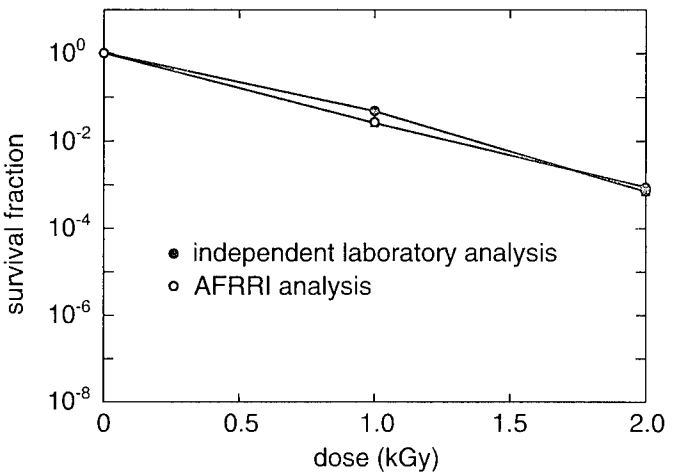

Fig. 3. *Survival fractions of dry* B. pumilus *spores after selected doses of neutron radiation; validation of the AFRRI technique used for spore survival analyses after neutron irradiation compared to analyses performed by the independent commercial laboratory. The filled circle (●) represents survival data determined by the independent commercial laboratory, and the open circle (O) represents survival data determined by AFRRI. Each data point represents spore survival obtained from 2-11 cellulose fiber spore-impregnated strips.*

Similar validation analyses were done comparing survival findings of both laboratories for γ-ray and neutron irradiated *B. subtilis* spores. Doses of γ radiation ranged up to 3 kGy (Fig. 4) and up to 2 kGy for neutrons (Fig. 5). In both laboratories, a 3-log kill recorded for γ-ray irradiation, and a 4-log kill was recorded for neutron irradiation. No statistically significant differences between the laboratories were found for survival after either irradiation.

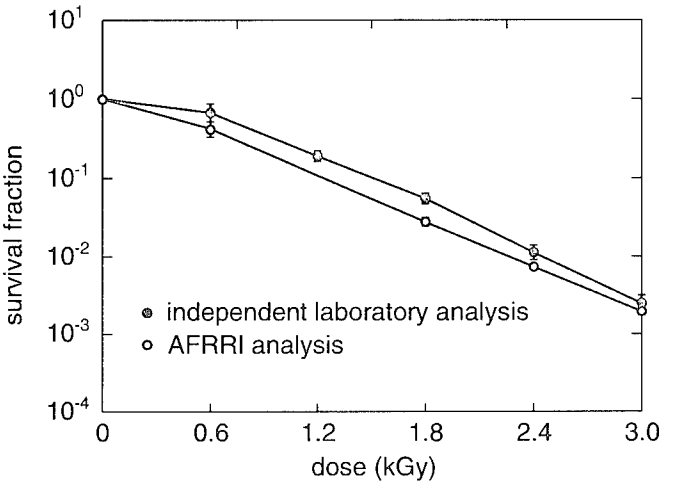

Fig. 4. *Survival of dry* B. subtilis *spores after γ irradiation. The filled circle (◎) represents survival data determined by the independent commercial laboratory, and the open circle (O) represents survival data determined by AFRRI. Each data point represents spore survival from 3-13 cellulose fiber spore-impregnated strips.*

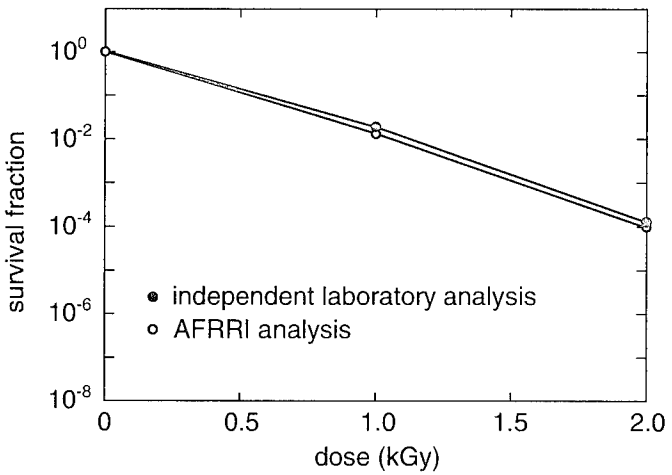

Fig. 5. *Survival of dry* B. subtilis *spores after neutron irradiation. The filled circle (●) represents survival data determined by the independent commercial laboratory, and the open circle (O) represents survival data determined by AFRRI. Each data point represents spore survival from 3-13 cellulose fiber spore-impregnated strips.*

Discussion

We developed a modified technique for quantifying the killing of spores after kGy doses of fission neutrons or γ-irradiation by using a readily available, commercially prepared matrix with a certificated bioburden. This technique was modified from the standard procedure (17) used to determine sterility after irradiation of foodstuffs and health-related products. The modified USP technique (17), used in conjunction with generally accepted instrumentation (6) for the measurement of fission spectrum neutrons and γ irradiation, demonstrated the killing of radiosensitive *B. subtilis* and radioresistant *B. pumilus* spores. In this study, fission neutrons were more effective in killing spores from both species than γ-rays. The spore-killing effectiveness of fission neutrons compared to γ-rays will be examined more fully in a subsequent publication. However, in the present study, the major dosimetric improvement, over that of previous workers (8, 16), consisted of our ability to accurately estimate the neutron and γ-radiation dose components of the total dose received by the spore samples exposed in the mixed radiation field of the AFRRI TRIGA reactor. This was possible because we used the paired ion chamber technique (6) for mixed-field

dosimetry in conjunction with data validated by the National Institute of Standards and Technology on the characteristics of standard neutron exposure fields available in the AFRRI reactor (14).

The quantitative microbiologic and radiation dosimetric techniques in this report may be used to demonstrate radiation-related spore killing in a variety of environmental conditions. However, in this report, the matrix containing the spores was irradiated while dry. In preliminary experiments, not reported here, the quantitation techniques described were modified to evaluate radiation-induced spore kill on moist cellulose fiber strips.

A "tailing" effect was found with bacterial spores of *B. pumilus* irradiated with 12-15 kGy of γ rays. The few colonies that formed were positively identified as *Bacillus* species. This tailing phenomenon may be due to resistance to radiation, to extremely low grade contamination with spores or vegetative forms of other microorganisms present in the laboratory environment, or to other variables as previously reviewed (3). While resistance of *B. pumilus* spores to high γ-radiation doses is a possibility, we found, in work not included in this report, that no colonies formed after spore-impregnated strips were irradiated with 18 kGy. This suggests that higher γ-radiation doses abrogate the possible resistance to radiation. Contamination by the environment was monitored by placing open agar plates on the laboratory bench for 1 hr during pour plating procedures. No colonies formed, suggesting that airborne contamination with spores or other vegetative bacteria was not a significant problem.

Resistance of spores to radiation is dependent on several factors, two of which were used in this study to develop the quantification assay. We developed and tested the technique by using two types of radiation and spores from two bacterial species. Other factors that contribute to spore survival after irradiation, such as dose rate, moisture, atmospheric conditions, nutritional status and pH of growth media, and age of irradiated spore populations, were not examined in this present work.

The matrix used to contain spore populations during irradiation was a precisely sized cellulose fiber strip. Further research will determine if a container or storage device having a greater bioburden (18), hence a greater concentration of spores, would be neutralized by radiation to the same extent as that reported here for spore-impregnated cellulose fiber strips.

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